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FINAL REPORT, NASA CONTRACT R-63

"Freezing and Drying of Living Cells"

This summary covers the period from September 1, 1962 to June 2, 1963. Although the formal date of initiation of this contract was June 1, 1962, funds were not actually received until late August. The contract has been extended on a no-cost basis to August 31, 1963.

The major portion of this contract is concerned with basic research studies on the mechanism of freezing and drying injury. There are a number of living organisms which are naturally resistant to freezing and drying and it was the purpose of this research to investigate the mechanism by which these organisms achieve their resistance and thereby to gain some insight into means for artificial protection of tissue against freezing injury. There is a certain degree of hazard implicit in this particular investigation since it is probable that many organisms are not actually resistant in the literal sense but rather have developed devices for avoiding or preventing freezing or drying. An example is found among certain insects which supercool at sub-freezing temperatures and do not form ice crystals within their tissues until extremely low temperatures are reached. There are many examples of organisms that protect themselves by drying through the formation of an impermeable epidermis or cuticle which prevents the loss of body water even under conditions of extremely low humidity. A vital aspect of a study of resistant organisms is, therefore, the choice of an organism in which internal ice formation does take place and in which exposure to drying does result in an appreciable loss of body water.

A number of nematodes have been studied by others. Certain nematodes are clearly able to survive both freezing and drying conditions in nature. Many species also exist which are sensitive to both of these conditions. There have been no studies of the nature of nematode resistance to drying although at least one author (Asahina) has demonstrated the presence of ice crystals within the bodies of nematodes which subsequently survive the freezing. The choice of nematodes as an experimental material was based on the hope that comparisons between resistant or susceptible species could be made. Particularly influencing this choice was the personal interest of Dr. Mary Burns who assumed responsibility for the nematode preparations and the freezing and drying experiments.

The first prerequisite for the use of nematodes as specimen material was their maintenance in laboratory culture and the preparation of clean and concentrated material for study. The bacteria-eating nematodes could be grown in culture in conventional fashion but the plant eating nematodes presented somewhat of a problem. Studies were undertaken on the preparation of plant cultures to support these organisms in the laboratory. Cultures of plant

cells from the sycamore, Ditylenchus dipsaci, were prepared and cultures were also successfully preserved by freezing in the presence of dimethylsulfoxide. It proved possible to prepare concentrated and relatively pure suspensions of nematodes, although phased cultures with a high degree of uniformity in age were not achieved.

The existence of a considerable age distribution in the nematode cultures proved to be one of the major drawbacks to their use for studies of freezing and drying resistance. The nematode, Panagrellus redivivus, showed a strong correlation between age and resistance. Older forms showed little or no resistance to freezing and drying while younger forms were increasingly resistant. In many cases gravid females were killed by freezing or drying while their ova survived to hatch and grow normally. Furthermore, resistance was not found to be consistent even in a relatively homogeneous population. This is particularly true as one indulges in the more severe exposures which would be of greater scientific interest. Inasmuch as it was our original intent to investigate characteristics of histochemistry and microstructure associated with resistance or susceptibility to freezing, this created a major problem since it prevented the study of an individual organism in detail with any confidence regarding its expectation of resistance or susceptibility.

Concurrent with the exploratory studies briefly outlined above, the necessary techniques for the study of these organisms were under development. Our histologist investigated a large variety of histochemical stains for their applicability to the nematode. A biologist was trained in and undertook the development of suitable procedures for the thin sectioning and electron staining of nematode preparations. Both of these technical studies, the histochemical and the electron microscopical, were competently conducted and resulted in the establishment of techniques not hitherto reported.

A collaborative relationship has been established with Dr. Chitwood of the Department of Agriculture for the study and evaluation of the control sections that have been prepared so far. Since these preparations reveal structures and staining characteristics not hitherto observed, Dr. Chitwood is extremely interested in assisting with the interpretations and analyses of our preparations.

In pursuing the light and electron microscopical investigations, the sparseness of the existing literature on nematode anatomy, particularly at the electron microscopical level, became increasingly apparent. A very large amount of groundwork will have to be laid to establish in advance the definitive micro-anatomy of our specimens before any well-controlled study of departures from normal can be undertaken.

The various obstacles listed above: difficulty of growing phased cultures, non-homogeneity of nematode populations with regard to freezing or drying injury and lack of adequate background in the literature relating to nematode microstructures, all conspire to render difficult the use of the nematode as an experimental subject. Above all, Dr. Mary Burns' departure has left us with the problem of specimen preparation and the lack of an individual specifically interested in and familiar with nematodes.

At the present time, a series of experiments are being undertaken to determine whether the effects on these organisms of freezing and drying as studied by light and electron microscopy is of sufficient promise to warrant wrestling with the various obstacles that their handling entails. Unless these preliminary studies indicate unusual experimental opportunity, our attention will be shifted to a more suitable specimen material.

Preliminary studies are now being undertaken of the intertidal mollusks as a substitute for the nematodes for the study of freezing injury and resistance to freezing. The mollusks which occupy the intertidal areas during the winter are subjected to freezing twice daily, in northern regions, to temperatures below -30°C. These organisms appear to offer several advantages over the nematode. Since their freezing resistance is complete and uniform, individuals can be studied with complete confidence of their anticipated behavior. Their large size will considerably facilitate measurements of water content, ice formation, metabolism and other physical or physiological events of interest.

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